Atty. Dkt. 1579-880
1767 DIV

U.S. PATENT APPLICATION

Inventor(s): Barton F. HAYNES
Hua-Xin LIAO
Norman LETVIN

Invention: HUMAN IMMUNODEFICIENCY VIRUS VACCINE

NIXON & VANDERHYE P.C.
ATTORNEYS AT LAW
1100 NORTH GLEBE ROAD, 8TH FLOOR
ARLINGTON, VIRGINIA 22201-4714
(703) 816-4000
Facsimile (703) 816-4100

SPECIFICATION

HUMAN IMMUNODEFICIENCY VIRUS VACCINE

This is a continuation-in-part of Application No. 09/497,497, filed February 4, 2000, now pending, the entire contents of which is incorporated herein by reference.

TECHNICAL FIELD

The present invention relates, in general, to human immunodeficiency virus (HIV) and, in particular, to an HLA-based HIV vaccine.

BACKGROUND

As the HIV epidemic continues to spread world-wide, the need for an effective HIV vaccine remains urgent. The extraordinary ability of HIV to mutate, the inability of many currently known specificities of anti-HIV antibodies to consistently neutralize HIV primary isolates, and the lack of a complete understanding of the correlates of protective immunity to HIV infection have impeded efforts to develop an HIV vaccine having the desired effectiveness.

Although a majority of HIV-infected subjects develop acquired immunodeficiency syndrome (AIDS), approximately 10-15% of patients are AIDS-free after 10 years of infection, and are termed non-progressors to AIDS (Sheppard et al, AIDS 7:1159-66 (1993), Phair, AIDS Res. Human Retroviruses 10:883-885 (1994)). Of those that do develop AIDS,

those that do develop AIDS, approximately 10% of HIV-infected patients progress to AIDS within the first two to three years of HIV infection, and are termed rapid progressors to AIDS (Sheppard et al, AIDS 7:1159-66 (1993), Phair, AIDS Res. Human Retroviruses 10:883-885 (1994)). The initial characterization of anti-HIV immune responses in non-progressors and rapid progressors to AIDS has provided some insight into what may be the correlates of protective immunity to HIV.

In general, rapid progressors to AIDS have lower levels of antibodies to HIV proteins (Sheppard et al, AIDS 7:1159-66 (1993), Pantaleo et al, N. Engl. J. Med. 332:209-216 (1995), Cao et al, N. Eng. J. Med. 332:201-208 (1995)), and low or absent antibodies that neutralize autologous HIV isolates (Pantaleo et al, N. Engl. J. Med. 332:209-216 (1995), Cao et al, N. Eng. J. Med. 332:201-208 (1995)). Anti-HIV CD8+ CTL activity is present in peripheral blood T cells of rapid progressors, although one study has found low levels of memory CD8+ CTL by precursor frequency analysis in rapid progressors versus non-progressors (Pantaleo et al, Nature 370:463-467 (1994), Rinaldo, personal communication (1995)). Plasma levels of HIV virions are generally higher in rapid progressors compared to non-progressors, and rapidly replicating HIV strains are isolated more frequently from rapid progressors (Lee et al, J. AIDS 7:381-388 (1994),

Mellors et al, Ann. Intern. Med. 122:573-579 (1995), Jurriaans et al, Virology 204:223-233 (1994)), either as a consequence of immunodeficiency and selection of more virulent HIV variants, or as a consequence of more virulent HIV variants infecting rapid progressors (Sullivan et al, J. Virol. 69:4413-4422 (1995)). Taken together with data that the fall in plasma viremia in primary HIV infection correlates with the presence of CD8+ anti-HIV CTL activity (Borrow et al, J. Virol. 68:6103 (1994)), these data suggest that anti-HIV CD8+ CTL that kill HIV-infected cells and antibodies that broadly neutralize HIV primary isolates, might be protective anti-HIV immune responses in uninfected individuals subsequently exposed to HIV (Haynes et al, Science 271:324-328 (1996), Haynes, Science 260:1279-1286 (1993)).

It has been suggested that less effective anti-HIV CD8+ CTL responses may be oligoclonal regarding TCR Vβ usage and targeted at several non-immunodominant HIV CTL epitopes, whereas more effective anti-HIV CTL responses may be polyclonal and targeted at fewer immunodominant epitopes (Rowland-Jones et al, Nature Medicine 1:59-64 (1995), Nowak et al, Nature 375:606-611 (1995)). Taken together with data that suggest the inheritance of certain HLA-encoded or other host genes may be associated with either rapid progression or non-progression to AIDS (Haynes et al, Science 271:324-328 (1996)), these data suggest that host gene

expression may determine the *quality* and/or *quantity* of host anti-HIV immune responses.

Potent non-HLA restricted CD8+ T cell anti-HIV activity that suppresses the ability of HIV to replicate has been described by Levy et al (Walker et al, Science 234:1563-1566 (1986)). This CD8+ "HIV suppressor" activity is initially present in rapid progressors, then declines with the onset of AIDS (Walker et al, Science 234:1563-1566 (1986)), and may be mediated in part by cytokines such as IL-16 (Baier et al, Nature 378:563 (1995)), and by the chemokines, RANTES, MIP-1a and MIP-1b (Cocchi et al, Science 270:1811-1815 (1995)). Berger and colleagues have recently discovered a novel host molecule termed *fusin*, that is required for T cell tropic HIV to infect CD4+ T cells, and has significant homology with a known chemokine receptor, the IL8 receptor (Feng et al, Science 272:872-877 (1996)).

Thus, for induction of CD8+ "HIV suppressor" cells, CD8+ CTL and CD4+ T helper cells by an HIV immunogen, what is most likely needed are immunogens that induce these anti-HIV responses to a sufficient number of HIV variants such that a majority of HIV variants in a geographic area will be recognized.

A key obstacle to HIV vaccine development is the extraordinary variability of HIV and the rapidity and extent of HIV mutation (Win-Hobson in The Evolutionary biology of Retroviruses, SSB Morse Ed. Raven Press, NY, pgs 185-209 (1994)). Recent data in patients treated

with anti-retroviral drugs have demonstrated that HIV variants emerge rapidly after initiation of treatment and can be isolated from peripheral blood as early as 3 weeks after initiation of drug treatment (Wei et al, Nature 373:117-122 (1995), Ho et al, Nature 373:123 (1995)). Moreover, up to 10^9 new HIV virions are produced in an infected individual per day, and the half-life of HIV quasispecies is approximately 2 days (Wei et al, Nature 373:117-122 (1995), Ho et al, Nature 373:123 (1995)).

Myers, Korber and colleagues have analyzed HIV sequences worldwide and divided HIV isolates into groups or clades, and provided a basis for evaluating the evolutionary relationship of individual HIV isolates to each other (Myers et al (Eds), Human Retroviruses and AIDS (1995), Published by Theoretical Biology and Biophysics Group, T-10, Mail Stop K710, Los Alamos National Laboratory, Los Alamos, NM 87545). The degree of variation in HIV protein regions that contain CTL and T helper epitopes has also recently been analyzed by Korber et al, and sequence variation documented in many CTL and T helper epitopes among HIV isolates (Korber et al (Eds), HIV Molecular Immunology Database (1995), Published by Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545).

A new level of HIV variation complexity was recently reported by Hahn et al. by demonstrating the frequent recombination of HIV among clades (Robinson et al, J. Mol. Evol. 40:245-259 (1995)). These authors suggest that as many as 10% of HIV isolates are mosaics of recombination, suggesting that vaccines based on

only one HIV clade will not protect immunized subjects from mosaic HIV isolates (Robinson et al, J. Mol. Evol. 40:245-259 (1995)).

The large number of HIV variants available for transmission and the possible immunodominant nature of what may be protective anti-HIV T cell responses has suggested the need for consideration of development of HLA-based HIV subunit vaccines (Palker et al, J. Immunol. 142:3612-3619 (1989), Berzofsky, FASEB Journal 5:2412 (1991), Haynes et al, Trans. Assoc. Amer. Phys. 106:33-41 (1993), Haynes et al, AIDS Res. Human. Retroviral. 11:211 (1995), Ward et al, In Lost Alamos Database (1995), B. Korber (Ed). In press, Cease et al, Ann. Rev. Immunol. 12:923-989 (1994)). The present invention provides such a vaccine.

SUMMARY OF THE INVENTION

The present invention relates to an HLA-based vaccine against HIV. Vaccines of the invention, which induce salutary anti-HIV immune responses, can be designed based on analysis of the HLA alleles present in the cohort to be immunized and analysis of the most common HIV variants present in the geographic location of the cohort. The invention also relates to a method of immunizing a patient against HIV using the HLA-based vaccine.

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D. C4-V3 Th-CTL Peptides Induce HLA B7 Reactive CD8+ CTL in Normal HIV-1 Seronegative Humans. Figs. 1A and 1C show specific lysis from *in vivo* immunization and *in vitro* restimulation against each of the V3 B7 CTL epitope variants. BLCL=B lymphoblastoid cell (BCLC) no peptide coating control. C4=C4 Th determinant peptide on BCLC, V3MN, V3RF, V3EV91, and V3Can0A are the B7 CTL epitope variant peptide coated on BCLC. Data show patient in Fig. 1A responded to 1 of 4 B7 CTL epitope variants (the HTV EV91 variant) while the patient in Fig. 1C responded to 3 of 4 B7 epitope variants (HIV MN, EV91 and Can0A). Figs. 1B and 1D show 2 HLA B7 negative individuals that made no CTL response to the B7-restricted CTL peptide immunogen after both in *in vivo* immunization and *in vitro* restimulation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an HLA-based HIV vaccine. The invention further relates to a method of immunizing a patient against HIV by using such a vaccine.

The HLA-based vaccines of the invention can be designed based on available HLA databases. Results obtained in International Histocompatibility Testing Workshops, such as the most recent ones

(Histocompatibility Testing 1980, Teresaki (Ed.), UCLA Tissue Typing Laboratory, Los Angeles, CA (1980), Histocompatibility Testing 1984, Albert et al (Eds.), Springer-Verlag, Berlin (1984), Immunobiology of HLA, 2 volumes, Dupont (Ed.), Springer-Verlag, New York, (1989), HLA 1991, 2 volumes, Tsuji et al (Eds.), Oxford University Press, Oxford (1992)), provide such a database.

The International Histocompatibility Workshop data (such as Histocompatibility Testing 1984, Albert et al (Eds.), Springer-Verlag, Berlin (1984), HLA 1991, 2 volumes, Tsuji et al (Eds.), Oxford University Press, Oxford (1992)), supplemented with published data from selected laboratories (such as Williams et al, Human Immunol. 33:39-46 (1992), Chandanayingyong et al, In Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference, Simons et al (Eds.), Immunopublishing, Toorak, pgs. 276-287 (1983)) provide an estimate of the frequencies of HLA alleles that have been shown to serve as restriction elements for HIV CTL epitopes (HIV Molecular Immunology Database (1995), Korber et al (Eds.), Los Alamos National Laboratory: Published by Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545). Table 1 summarizes these frequencies for the four populations: African Americans, North American Indians, USA Caucasians, and Thais, used here for purposes of exemplification.

Section II of the Los Alamos HIV epitope database of Korber et al (HIV Molecular Immunology Database (1995), Los Alamos National Laboratory: Published by Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545) lists the CTL epitopes by HLA restriction element. Using these two sets of data and the Hardy-Weinberg theorem (Hardy, Science 28:49-50 (1908)), the proportion of each of the four populations that would be predicted to present peptides to the immune system if a limited number of HIV epitopes were included in a vaccine designed specifically for that population can be estimated. A similar calculation for a vaccine designed to be immunogenic for all four populations has been made. These results are presented in Table 2.

The strategy that can be used in this analysis is to first identify the most frequent restriction elements in the population under consideration for vaccination (or common to the 4 populations), to identify peptides that are presented by more than one HLA allele, and then to seek commonality between these two lists. Probability calculations then utilize the frequencies of the commonality alleles supplemented by those of additional high frequency alleles in the population. Alleles can be added until the proportion of the individuals in the population carrying one or more of the alleles in the list is at

an acceptable level, for instance, greater than 90% in the examples. The aim is to maximize the sum of the HLA gene frequencies that recognize the least number of different HIV peptides to be included in an HIV immunogen. The next step is to choose the peptides associated with the restricting allele. In some instances,only one peptide is associated with an allele while in others, multiple peptides are presented by the same allele.

Criteria that can be used choosing which immunogenic epitopes to be included in a preventive HIV immunogen are listed below:

1. Peptides reported to be immunogenic in situations thought to reflect protection from retroviral infection or protection from retroviral-induced immunodeficiency disease (ie, in non-progressors to AIDS).

2. Peptides presented to the immune system by HLA restricting elements reported to be associated with non progression to AIDS (for example, Haynes et al, Science 171:324-328 (1996)).

3. Peptides reported to be "immunodominant" stimulators of HLA class I-restricted anti-HIV CTL responses (Nowak et al, Nature 375:606-611 (1995)).

4. Peptides reported presented by several disparate HLA class I allotypes.

For the four population cohorts considered in detail here by way of example, as few as 2 and as many as 5 epitopes are required to achieve a theoretical protection level of at least 90% (Table 2). The different numbers of required epitopes reflect the relative amounts of HLA Class I polymorphism observed in the different ethnic groups and presentation of a peptide by multiple HLA class I molecules. To date, HIV peptides have been associated only with HLA restriction elements that are infrequent in some populations. As more data are accumulated for other epitopes, some that are associated with higher frequency restriction elements may be identified.

A comparison between the individual and combined populations (Table 2) demonstrates that relatively little is gained by including epitopes that are associated with low frequency alleles. The proportion of individuals protected approaches 100% asymptotically so that even adding on epitopes associated with high frequency alleles adds little to the proportion as this level is approached. This is illustrated by the North American Indians where including 6 more epitopes associated with 5 very low frequency alleles and one intermediate frequency allele in the combined theoretical vaccine adds only 3.0% protection.

USP 5,993,819 (the contents of which is incorporated herein by reference) also includes a

description of the steps involved in the development of an HLA-based HIV vaccine. In Table XXVI of that patent, the following vaccine formula is provided which is equally applicable here:

$$Th_1-X_1,\ Th_2-X_2,\ Th_3-X_3,\ \ldots\ Th_N-X_N$$

where Th = immunodominant T helper epitopes and X = MHC Class I CTL epitopes. In the context of a preferred embodiment of the invention, Table 3 provides specific TH-X peptides (see vaccines 6, 8 and 10, particularly vaccines 6 and 8) that can be admixed, formulated with a pharmaceutically acceptable carrier, and adjuvant, as appropriate, and administered to a patient in order to effect immunization. The optimum amount of each peptide to be included in the vaccine and the optimum dosing regimen can be determined by one skilled in the art without undue experimentation.

As an alternative to using mixtures of individual Th-X peptides, the vaccine of the presently preferred embodiment can also take the form of a linear array of Th-X epitopes (see the linear arrays of MVA 6-10 in Table 4, particularly MVA 6 and MVA 8), preferably, expressed in a modified *Vaccinia ankara* (Zentralbl. Bakterial 167:375-390 (1978); Nature Med. 4:397-402 (1988)) or other live vector such as an adenoviral vector or a canary pox vector (Weinhold et al, Proc. Natl. Acad. Sci. 94:1396-1401 (1997)). Upon expression with HIV gag p55, pseudovirons (particles)

are produced (see, for example, the linear arrays of MVA 7 and 9 in Table 4). Standard procedures can be used to formulate the vaccine (e.g., with a carrier and, as appropriate, with an adjuvant) and optimum dosing regimes can be determined by one skilled in the art without undue experimentation.

In a further embodiment, the vaccine of the present invention includes MHC Class I restricted cytotoxic T lymphocytes (CTL) epitopes from HIV p17 and p24 gag regions. Known HIV CTL epitopes and their MHC restricting elements are listed in "HIV Molecular Immunology Database, 1999" (Korber, BTM, Brander, C., Haynes, B.F. et al Editors, Published by the Theoretical Biology and Biophysics Group T-10, Mail Stop K710 Los Alamos National Laboratory, Los Alamos, New Mexico 87545). The CTL regions designated CTL-J, CTL-K, CTL-L and CTL-M are selected for Vaccine 11 in Table 3. The full peptide has been designed to have at the N-terminus of the epitope the optimal Th determinant, ThA E9V from HIV gp120 C4 region. The restricting elements predicted to respond to these peptides are listed to the right in Table 3. Thus, a practical HIV gag CTL immunogen is set forth in Table 6, with A-Th/A-CTL and B-Th/B-CTL peptides mixed with the peptides in Vaccine 11. The 25 HLA Class I molecules predicted to recognize the peptides in the mixture of peptides in Table 6 are listed at the bottom of the table.

Complex immunogens made up of CTL sequences, for example, from the Los Alamos Database (Korber, BTM, Brander, C., Haynes, B.F. et al Editors, Published by the Theoretical Biology and Biophysics Group T-10, Mail Stop K710 Los Alamos National Laboratory, Los Alamos, New Mexico 87545) can be prepared by adding to the sequences in Table 6, new sequences from CTL epitopes in envelop, rev, nef, tat, pol and other regions of the HIV genome. These sequences can be formulated with T helper sequences as above in Table 6 (generic Th-X1, Th-X2......Th-Xn), or can be delivered in shorter sequences of X1,X2,......Xn, with T cell help being delivered by an appropriate adjuvant. In these generic designs, Th represents a helper T cell epitope, and X represents a HLA Class I restricted CTL epitope.

At each CTL sequence, there are many variants that can be included in the peptide mix in the above vaccine designs, in order to provide CTL that attack a sufficient number of HIV variants to prevent infection or to control infection. Variants are listed for each HIV Clade in the Los Alamos database for HIV sequences, "Human Retroviruses and AIDS", Kuiken, C, Foley, B et al Editors, Published by the Theoretical Biology and Biophysics Group T-10, Mail Stop K710 Los Alamos National Laboratory, Los Alamos, New Mexico 87545.

Since different geographic locations around the world have different HIV Clades infecting patient cohorts, the above peptide design can be modified to be appropriate for the Clade or Clades of HIV that are relevant for a particular geographic region. For example, the Los Alamos Database of HIV Sequences has a listing of sequences by country and by clade. Therefore, to design a CTL vaccine for Zambia in Sub-saharan Africa, the principles and general CTL epitope design described as above can be employed but using the most common or consensus sequences of the Clades and isolates in the data base from Zambia. This general strategy applyies to design of CTL immunogens for any geographic region of the world.

Peptides have the greatest use in focusing the immune response on many dominant and subdominant CTL epitopes of HIV, but may benefit from a prime from another type of immunogen. Thus, the sequences described above and given in Tables 3 and 6, as well as Zambian sequences and or sequences of epitopes from rev, nef, tat, pol or env, can also be constructed in linear arrays of CTL epitopes with or without T helper determinants, for example, in either plasmid DNA constructs or in live vector constructs such as Modified Vaccinia Ankara or in mycobacteria tuberculosis strains that are attenuated, such as BCG (Jacobs et al, Nature Medicine 2:334 (1996)). These DNA or live vectors with linear arrays of CTL epitopes

can be used as either primes or boosts of peptides or of each other to optimally give CTL anti-HIV responses.

It will be appreciated that this embodiment of the invention includes not only the specific Th-X peptides, and derivatives thereof (e.g. as shown in MVA 7 and MVA 9 in Table 4), shown, for example, in Tables 3 and 4, but also includes variants of the indicated peptides as well, particularly variants of the CTL epitopes shown. The mixture or linear array of Th-X peptides can be used alone or as one component of a multi-component vaccine. It will also be appreciated that the peptides of the invention can be synthesized using standard techniques. It will also be appreciated that the vaccine of the present invention can take the form of a DNA vaccine the expression of which *in vivo* results in the expression of the peptides, or linear arrays of same, described above.

Suitable routes of administration of the present vaccine include systemic (e.g. intramuscular or subcutaneous). Alternative routes can be used when an immune response is sought in a mucosal immune system (e.g., intranasal). Appropriate routes and modes of administration can be selected depending, for example, on whether the vaccine is a peptide or DNA vaccine or combination thereof.

Certain aspects of the present invention are described in greater detail in the Example that follows.

EXAMPLE 1

Studies of Th-CTL Mutivalent in HLA B7+ Humans

Immunogenicity and Safety of the C4-V3 Th-CTL Polyvalent Immunogen in HIV Seropositive Patients with CD4+ T Cell Counts >500/mm3 (DATRI010). The DATRI010 human trial of the C4-V3 PV immunogen has been completed (Bartlett et al, AIDS Res. Hum. Retro. 12:1291-1300 (1998)). The immunogen was 4 Th-CTL peptides with the Th epitope the same in each peptide and the CTL peptide was four variants of a B7-restricted env CTL epitope (Haynes, Res. Human Retro. 11:211-221 (1995), Beddows et al, J. Gen. Virol. 79:77-82 (1998), Table 5). Ten HIV-infected, HLA B7-positive patients with CD4+ T cells >500/mm3 were enrolled. Eight patients received 2 mg of C4-V3 polyvalent immunogen (ie, 500 μg of each peptide) emulsified in incomplete Freund's adjuvant (Seppic ISA51) IM X5 over 24 weeks, and 2 controls received ISA51 IM alone. Vaccine recipients had excellent boosts of Th proliferative levels and neutralizing antibody levels to TCLA HIV (Bartlett et al, AIDS Res. Hum. Retro. 12:1291-1300 (1998)). However, in the setting of HIV infection, PBMC suspensions of

immunized B7+ subjects had minimal direct CTL activity to the B7-restricted *env* CTL epitope in the immunogen to peptide coated targets or to vaccinia infected targets (i.e. the B7 gp120 CTL epitope was non-dominant in the setting of HIV infection) (Bartlett et al, AIDS Res. Hum. Retro. 12:1291-1300 (1998)).

AVEG020 Trial of Th-CTL C4-V3 Peptides in Seronegative Subjects. In conjunction with NIAID, DAIDS, DATRI and WLVP, AVEG020 "Phase 1 Safety and Immunogenicity Trial of C4-V3 Peptide Immunogen in HIV Seronegative Subjects" was carried out at Vanderbilt, Rochester, and Seattle as a multicenter trial (AVEG020 Doses: High Dose = 4 mg total dose, 1 mg of each peptide per dose; Low Dose = 1 mg total dose, 250 μg of each peptide per dose).

Studies were made of 13 subjects (9, B7- and 4 B7+) after two immunizations 250 μg of each peptide variant. Of 9 HLA B7-subjects, 0/9 had PB CTL activity to any of the peptide variants of the B7-restricted gp120 *env* CTL epitope in the immunogen (Figure 1B and 1D). In contrast, 2/4 HLA B7+ subjects had high levels of CTL activity to the B7 epitope that was mediated by CD8+ T cells and was MHC restricted after only two immunizations (Figure 1A and 1C). These data provided direct evidence that Th-CTL immunogens, when formulated in potent adjuvants, could induce MHC Class I-restricted CATL in humans. Whereas one subject responded to one of the 4 B7 epitope

variants, the other subject (Figure 1A) responded to 3 of the 4 CTL variants. These data demonstrated that a human host could respond to more than one CTL epitope variant in an immunogen, and indicated that epitope-based immunizations could be used to induce MHC Class I-restricted CD8+ CTL responses to CTL epitopes and to their variants.

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All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

Table 1 Frequencies of HLA Class I Alleles That are Known to Serve as HIV CTL Restriction Elements in Four Populations

HLA Alleles	Frequencies* African Americans	USA Caucasians	North American Indians	Thais
A2	16.7	28.3	25.5	25.5
A3	8.9	12.2	2.9	1.5
A11	2.3	5.5	1.0	32.5
A24	4.7	9.6	19.6	14.6
A28	10.9	4.5	6.9	0.8
A30	9.5	2.6	2.0	1.1
A31	1.7	2.0	27.5	1.7
A32	1.0	5.1	2.0	0.2
A33	8.1	1.0	1.0	13.6
B7	8.3	10.0	3.9	2.7
B8	3.2	10.0	5.6	0.2
B12 (44)	6.2	10.4	3.9	5.4
B13	0.9	3.0	1.0	9.3
B14	3.0	4.1	2.9	0.4
B17	10.9	4.9	1.0	8.1
B18	3.3	4.9	1.0	2.5
B27	1.6	4.1	2.9	6.0
B35	7.7	8.5	18.6	2.5
B37	0.9	2.2	0.0	1.4
B52	1.1	1.2	2.9	3.1
B53	12.8	0.8	0.0	0.0
B57	4.2	3.9	1.0	5.2
B60	1.3	4.5	2.9	8.3
B62	1.4	5.5	4.9	5.0
Cw3	9.6	12.6	22.4	15
Cw4	21.0	9.8	15.4	6

*Frequencies for HLA-A and HLA-B alleles are taken from HLA 1991 [21]. HLA-C for African Americans and USA Caucasians are taken from Histocompatibility Testing 1984 [19], HLA-C for North American Indians from Williams and McAuley, 1992 [22], and HLA-C for Thais from the Proceedings of the Second Asia and Oceania Histocompatibility Workshop Conference [23].

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Table 2 **Proportion of each of the four populations that would be predicted to present peptides to the immune system**

Population	HLA Restriction Elements Chosen	HIV Protein	Epitope Location	Epitope
a) African Americans	A2, A3, A11, B35	nef	73–82	QVPLRPMTYK
	A28, B14	gp41	583–592	VERYLKDQQL
	A30, B8	gp41	844–863	RRIRQGLERALL
	B17, B37	nef	117–128	TQGYFPQWQUYT
	Cw4	gp120	576–383	(S) FNCGGEFF
(Proportion of African Americans expected to present these 5 epitopes is 92.3%)				
b) USA Caucasians	A2, A3, A11, B35	nef	73–82	QVPLRPMTYK
	A30, B8	gp41	844–863	RRIRQGLERALL
	B7	gp120	302–312*	RPNNNTRKSI
		nef	126–138*	NYTPGPGVRYPLT
	B12	p24	169–184	IPMFSALSEGATPQDL
(Proportion of USA Caucasians expected to present these 4 epitopes is 90.2%)				
c) North American Indians	A2, A3, A11, B35	nef	73–82	QVPLRPMTYK
	A24	gp41	584–591*	YLKDQQL
		nef	120–144*	YFPDWQNYTPGPGIRYPLTFGWCYK
	A31	gp41	770–780	RLRDLLLIVTR
(Proportion of North American Indians expected to present these 3 epitopes is 96.4%)				
d) Thais	A2, A3, A11, B35	nef	73–82	QVLRPMTYK
	A24	gp41	584–591*	YLKDQQL
		nef	120–144*	YFPDWQNYTPGPGIRYPLTFCGWCYK
(Proportion of Thais expected to present these 2 epitopes is 93.6%)				
e) African Americans USA Caucasians	A2, A3, A11, B35	nef	73–82	QVPLRPMTYK
North American	A28, B14	gp41	583–592	VERYLKDQQL
Indians	A30, B8	gp41	844–863	RRIRQGLERALL
Thais	B17, B37	nef	117–128	TQGYFPQWQNYT
	Cw4	gp120	376–383	(S) FNCGGEFF
	B7	gp120	302–312*	RPNNNTRKSI
		nef	126–138*	NYTPGPGVRYPLT
	B12	p24	169–184	IPMFSALSEGATPQDL
	A31	gp41	770–780	RLRDLLLIVTR
	A24	gp41	584–591*	YLKDQQL
		nef	120–144*	YFPDWQNYTPGPGIRYPLTFCGWCYK

(Proportions of African Americans, USA Caucasians, North American Indians, and Thais expected to present these 9 epitopes are 95.4%, 97.5%, 99.4%, and 97.2%, respectively)

*The criteria upon which choices among peptides should be made are not yet known. It may be important to choose peptides that have been reported to be immunogenic in non-progressors to AIDS or that have been reported to induce immunodominant anti-HIV T-cell responses.

TABLE 3

Th-CTL Peptide Prototype Vaccine Immunogens for Testing in Either Mice, Rhesus Macaque or Human

Vaccine number	Name of Peptides	Species on which to be studied	Amino acid sequence	Restricting elements for CTL epitope	
1.	**Mouse HIV-1 Th-CTL epitopes**		Th - CTL		
	A-Th/A-CTL	Mouse	HAGPIAPGQMREPRG-KQIINMWQEVGKAMYA	H-2^{d}	14
	B-Th/B-CTL	Mouse	KEKVYLAWVPAHKGIG-MYAPPIGGQI	H-2 K^{d}	15
	C-Th/C-CTL	Mouse	QLLFIHFRIGCRHSR-DRVIEVVQGAYRAIR	H-2$^{[illegible]}$(D^{d})	16
	D-Th/D-CTL	Mouse	EQMHEDIISLWDQSL-RIHIGPGRAFYTTKN	H-2 D^{d}	17
3.	**Macaque SIV/HIV-1 Th-CTl epitopes**		Th - CTL		
	Th1/CTL/SIV Gag	Macaque	ELYKYKVVKIEPLGVAPTKA-CTPYDINQM	Mamu-A*01	18
	Th2/CTL/SIV Pol	Macaque	VSTVQCTHGIRPVVSTQLLL-STPPLVRL	Mamu-A*01	19
	Th3/CTL/HIV-1 Env	Macaque	STSIRGKVQKEYAFFYKLDI-YAPPISGQI	Mamu-A*01	20
5.	**Macaque SIV/HIV-1 Th-CTL p11c epitopes variants**		Th - CTL		
	Th1/CTL/SIV Gag	Macaque	ELYKYKVVKIEPLGVAPTKA-CTPYDINQM	Mamu-A*01	20D
	Th2/CTL/ SIV Gag/p11c/I-Y	Macaque	VSTVQCTHGIRPVVSTQLLL-CTPYDYNQML	Mamu-A*01	21
	Th3/CTL/ SIV Gag/p11c/I-A	Macaque	STSIRGKVQKEYAFFYKLDI-CTPYDANQML	Mamu-A*01	22
	Th4/CTL/ SIV Gag/p11c/I-D	Macaque	EYAFFYKLDIIPIDNDTTSY-CTPYDDNQML	Mamu-A*01	23
	Th5/CTL/ SIV Gag/p11c/I-K	Macaque	REQFGNNKTIIFKQSSGGDPE-CTPYDKNQML	Mamu-A*01	24
6.	**Human HIV-1 Th-CTL overlapping epitopes**		Th - CTL		
	A-Th/A-CTL	Human	KQIINMWQEVGKAMYA-KAFSPEVIPMF	HLA B57,B58	25
	B-Th/B-CTL	Human	YKRWIILGLNKIVRMYS-NPPIPVGEIYKRWI-ILGLNKIVRMYSPTSI	HLA B35,B8,B27,A33,Bw62,B52	26
	C-Th/C-CTL	Human	DRVIEVVQGAYRAIR-VGFPVRPQVPLRPMTYK	HLA A1,B7,B8,B35,A11,A2,A3,A31	27
	D-Th/D-CTL	Human	ASLWNWFNITNWLWY-WVYHTQGFFPDWQNYTP	HLA B7,B57,A1,B8,B18,B35	28
8.	**Human HIV-1 Th-dominant/ subdominant CTL epitopes**		Th - CTL		
	A-Th/E-CTL	Human	KQIINMWQEVGKAMYA-SLYNTVATL	HLA A2	29
	B-Th/F-CTL	Human	YKRWIILGLNKIVRMYS-KIRLRPGGK	HLA A3	30
	C-Th/G-CTL	Human	DRVIEVVQGAYRAIR-KRWIILGLNK	HLA B27	31
	D-Th/H-CTL	Human	ASLWNWFNITNWLWY-GGKKKYKL	HLA B8	32
	E-Th/I-CTL		MREPRGSKIAGTTST-ERYLKDQQL	HLA B14	33
10.	**Human HIV-1 Th-CTL p17 epitope (A2 Variants)**		Th - CTL		
	B-Th/E-CTL	Human	YKRWIILGLNKIVRMYS-SLYNTVATL	HLA A2	34
	C-Th/J-CTL	Human	DRVIEVVQGAYRAIR-SLFNTVATL	HLA A2	35
	A-Th/K-CTL	Human	QIINMWQEVGKAMYA-SLYNAVATL	HLA A2	36
	D-Th/L-CTL	Human	ASLWNWFNITNWLWY-SLYNTVAVL	HLA A2	37
	E-Th/M-CTL	Human	MREPRGSKIAGTTST-SLFNLLAVL	HLA A2	38

Vaccine number	Name of Peptides	Amino acid sequence	Restricting elements for CTL epitope	
11.	**Human HIV-1 Th-CTL overlapping epitopes**	Th - CTL		
	A*-Th/J-CTL	KQIINMWQVVGKAMYA-GQMVHQAISPRTLNAWVKVV	A2, A202, A5, B7, B14, B57, B5701, B5801, B02, Cw3	39
	A*-Th/K-CTL	KQIINMWQVVGKAMYA-ATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEW	A2, A25, A26, B7, B12, B14, B1402, B27, B39, B52, B53, B57, B58, B8101, Cw8, Cw0102	40
	A*-Th/L-CTL	KQIINMWQVVGKAMYA-GPKEPFRDYVDRFYKTLRAEQASQEVKNWMT	A2, A202, A5, A24, A2402, A25, A26, A33, B7, B8, B12, B14 B35, B39, B44, B52, B53Bw62, B27, B2705, B57, B5701, B70, B71, Bw62, Cw3, Cw8, Cw0401	41
	A*-Th/M-CTL	KQIINMWQVVGKAMYA-KIRLRPGGKKKYKLKHIVWGSEELRSLYNTVATLYCVHQRI	A1, A2, A3, A3.1, A03, A11, A23, A24, A0201, A2402, B8, B27, B42, B62, Bw62, Cw4	42

A*-Th=C4E9V

Table 4

Linear Array of Th-CTL Epitopes To Be Expressed in Modified Vaccinia Ankara

MVA-1) **HIV-1 mouse Th-CTL epitopes in**

A-Th/p24 (H-2 b) — A-CTL/gp120 (H-2 abf) — B-Th/RT (H2 dfk) — B-CTL/gp120 (H-2K d) —
HAGPIAPGQMREPRG--KQIINMWQEVGKAMYA----KEKVYLAWVPAHKGIG----MYAPPIGGQI-

— C-Th/vpr (H-2 d) — C-CTL/gp41 (H-2 dpuq)(D d) — D-Th/gp120(H-2 d) — D-CTL/gp120/(H-2D d)
--QLLFIHFRIGCRHSR---DRVIEVVQGAYRAIR----EQMHEDIISLWDQSL---RIHIGPGRAFYTTKN

MVA-2) **p55/gag + the same HIV-1 mouse Th-CTL epitopes in MVA-1**

MVA-3) **HIV-1/SIV Th-CTL epitopes in**

Th1/gp120/DRB*w201 — CTL/SIV Gag (Mamu-A*01) — Th2/gp120/DRB1*5406 — CTL/SIV Pol (Mamu-A*01) —
ELYKYKVVKIEPLGVAPTKA-------CTPYDINQM--------VSTVQCTHGIRPVVSTQLLL-----STPPLVRL-

— Th3/gp120 — CTL/HIV-1 Env (Mamu-A*01)
--STSIRGKVQKEYAFFYKLDI--------YAPPISGQI

MVA-4) **p55/gag + the same HIV-1/SIV Th-CTL epitopes in MVA-3**

MVA-5) **SIV Th-CTL p11c epitope variants in**

Th1/DRB*w201 — CTL/SIV Gag (Mamu-A*01) — Th2/DRB1*5406 — CTL/Gag/p11c/I-Y —
ELYKYKVVKIEPLGVAPTKA----CTPYDINQML-------VSTVQCTHGIRPVVSTQLLL----CTPYDYNQML-

— Th3/P14 — CTL/Gag/p11c/I-A — Th4/P15 — CTL/Gag/p11c/I-D —
-STSIRGKVQKEYAFFYKLDI---CTPYDANQML------EYAFFYKLDIIPIDNDTTSY------CTPYDDNQML-

— Th5/P33 — CTL/Gag/p11c/I-K
-REQFGNNKTIIFKQSSGGDPE----CTPYDKNQML

MVA-6) **HIV-1 human Th-CTL overlapping epitopes in**

A-Th/gp120//422-437 — A-CTL/p24/30-40 — B-Th/GTH1/130-146 — B-CTL/P24/121-150
KQIINMWQEVGKAMYA----KAFSPEVIPMF----YKRWIILGLNKIVRMYS----NPPIPVGEIYKRWIILGLNKIVRMYSPTSI-

— C-Th/gp41/317-331 — C-CTL/Nef/64-80 — D-Th/gp41/157-171 — D-CTL/Nef/111-127
--DRVIEVVQGAYRAIR---VGFPVRPQVPLRPMTYK---ASLWNWFNITNWLWY----WVYHTQGFFPDWQNYTP

Restricting elements for CTL epitopes:

A-CTL epitope=HLA B57/B58; B-CTL epitope=HLA B35/B8/B27/A33/Bw62/B52;
C-CTL epitope=HLA A1//B7/B8/B35/A11/A2/A3/A31); D-CTL epitope=HLA B7/B57/A1/B8/B18/B35.

MVA-7) **p55 gag +the same HIV-1 human Th-CTL overlapping epitopes in MVA-6**

MVA-8) **HIV-1 Thdominant/subdominant CTL epitopes in**

A-Th/C4/422-437 — E-CTR/p17/77-85(A2) — B-Th/GTH1/130-146 — F-CTL/p17/18-26(A3) — C-Th/gp41/317-331 —
KQIINMWQEVGKAMYA-----SLYNTVATL-----YKRWIILGLNKIVRMYS----KIRLRPGGK------DRVIEVVQGAYRAIR-

— G-CTLp24/131-140(B27) — D-Th/gp41/157-171 — H-CTLp17/24-31(B8) — E-Th/p24/96-110 — I-CTLgp41/74-82(B14)
--KRWIILGLNK-----ASLWNWFNITNWLWY-----GGKKKYKL------MREPRGSKIAGTTST----ERYLKDQQL-

MVA-9) **p55/gag + the same HIV-1 Th-dominant/subdominant CTL epitopes in MVA-8**

MVA-10) **HIV-1 Th-CTL A2 p17 epitope (A2 Variants) in**

B-Th/GTH1/130-146 — E-CTL/p17/77-85(A2) — C-Th/gp41/317-331 — J-CTL/P17/Consensus A — A-Th/C4/422-437 —
YKRWIILGLNKIVRMYS----SLYNTVATL------DRVIEVVQGAYRAIR----SLFNTVATL-------KQIINMWQEVGKAMYA-

— K-CTL/P17/RF — D-Th/gp41/157-171 — L-CTL/P17/Consensus F — E-Th/p24/96-110 — M-CTL/P17/V1525
--SLYNAVATL----ASLWNWFNITNWLWY-------SLYNTVAVL--------MREPRGSKIAGTTST-----SLFNLLAVL

Table 5

HIV Polyvalent C4-V3 Peptides Studied in Guinea Pigs. Primates Or In Humans	
Peptide	gp120 C4 Region gp120 V3 Region
C4-V3MN	KQIINMWQEVGKAMYA**TRPNYNKRKRIHIGPGRAFYTTK**
C4-V3RF	KQIINMWQEVGKAMYA**TRPNNNTRKSITKGPGRVIYATG**
C4-V3EV91	KQIINMWQEVGKAMYA**TRPGNNTRKSIPIGPGRAFIATS**
C4-V3CanOA	KQIINMWQEVGKAMYA**TRPHNNTRKSIHMGPGKAFYTTG**
C4E9G-V3RF	KQIINMWQ**G**VGKAMYA**TRPNNNTRKSITKGPGRVIYATG**
C4E9V-V3RF	KQIINMWQ**V**VGKAMYA**TRPNNNTRKSITKGPGRVIYATG**
C4K12E-V3RF	KQIINMWQEVG**E**AMYA**TRPNNNTRKSITKGPGRVIYATG**
Sequences from the Los Alamos Database.	

TABLE 6

Th-CTL Peptide Prototype Vaccine Immunogens derived from HIV-1 gag

Vaccine number	Name of Peptides	Amino acid sequence	Restricting elements for CTL epitope
	Human HIV-1 Th-CTL overlapping epitopes	Th - CTL	
6	A-Th/A-CTL	KQIINMWQEVGKAMYA-KAFSPEVIPMF	B57,B58
6	B-Th/B-CTL	YKRWIILGLNKIVRMYS-NPPIPVGEIYKRWIILGLNKIVRMYSPTSI	B35,B8,B27,A33,Bw62,B52
11	A*-Th/J-CTL	KQIINMWQVVGKAMYA-GQMVHQAISPRTLNAWVKVV	A2, A202,A5, B7, B14, B57, B5701, B5801, B02, Cw3
11	A*-Th/K-CTL	KQIINMWQVVGKAMYA-ATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEW	A2,A25, A26, B7, B12, B14, B1402, B27, B39, B52, B53, B57, B58, B8101, Cw8, Cw0102
11	A*-Th/L-CTL	KQIINMWQVVGKAMYA-GPKEPFRDYVDRFYKTLRAEQASQEVKNWMT	A2,A202,A5,A24,A2402,A25,A26, A33, B7, B8,B12, B14 B35,B39, B44, B52, B53Bw62, B27, B2705, B57, B5701, B70, B71,Bw62, Cw3, Cw8, Cw0401
11	A*-Th/M-CTL	KQIINMWQVVGKAMYA-KIRLRPGGKKKYKLKHIVWGSEELRSLYNTVATLYCVHQRI	A1,A2,A3, A3.1,A03, A11, A23, A24, A0201, A2402, B8, B27, B42, B62, Bw62, Cw4

A*-Th=C4E9V

<u>Summary of restracting elements for CTL eptopes in Vaccines A, B, J, K, L and M</u>

A: A1, A2 (02), (01), A3, A3.1, A5, A11, A23, A24 (02), A25, A26 and A33.

B: B7, B8, B12, B14 (02), B27 (05), B35, B39, B42, B44, B52, B53, B57 (01), B58 (01) B62 (w62), B70 and B71.

C: Cw3, Cw4, Cw0401 and Cw8.

HIV Th/CTL vaccine.ABJKLM